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Growth hormone function in strains of mice selected for large and small size

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SUMMARY

The gene (*dw*) causing hypopituitary dwarfism was transferred by repeated backcrosses into strains of mice differing genetically in growth-rate through previous selection. The dwarf mice lack growth hormone, and the purpose was to find out if the differences in growth-rate between the strains were in any part due to differences in their growth hormone status – amount of hormone or tissue sensitivity. In the absence of growth hormone, i.e. in the dwarfs, the strains still grew at different rates, proving that growth hormone status was not the only cause of their differences. The effect of substituting the *dw* gene was, however, greater in the large strain than in the unselected control, and less in the small strain than in the control. The growth differences between the strains were therefore in part due to growth hormone. Tissue sensitivity in the three strains was compared by their responses to graded doses of exogenous growth hormone. The large and control strains did not differ, but the small strain had a lower sensitivity. The results suggest that the increased growth-rate of the large strain is partly due to an increased amount, or activity, of its circulating growth hormone, while the reduced growth-rate of the small strain is in part due to a reduced sensitivity of its target organs to growth hormone.

INTRODUCTION

The purpose of the work described here was to find out if growth hormone is in any way responsible for the differences in growth-rate between strains of mice previously selected for large and for small size. A genetical method of 'hypophysectomy' was used to produce mice lacking growth hormone, and the growth of these mice was then measured, when untreated and when treated with exogenous growth hormone. To produce the mice lacking growth hormone, the hypopituitary dwarf gene (*dw*) was transferred by repeated backcrosses into the large and small strains and into unselected controls of intermediate size. The dwarf mutant has a virtual lack of acidophils in the anterior pituitary (Lewis, 1967), resulting in a lack of growth hormone and prolactin and a reduced level of thyrotropic hormone (Bartke, 1965). Garcia & Geschwind (1968) were unable to detect any growth hormone in the plasma of dwarf mice (< 1 ng/ml), but Sinha, Salocks & Vanderlaan

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(1975) found low but measurable amounts (10 ng/ml). The growth of dwarf mice is not restored to normal levels by exogenous growth hormone alone. Detailed conclusions about the growth hormone status of the selected strains therefore cannot be drawn, but we think nevertheless that qualitative answers to the questions can be obtained.

There are many ways in which genetic differences might affect growth hormone function (Shire, 1976). The experiments were designed to detect two types of difference that might be connected with the differences of growth-rate produced by the previous selection. First, the amount of circulating hormone might differ; and second, the sensitivity of the target organs might differ. The first question, then, was whether the selected strains still grow at different rates in the absence of pituitary hormones. If they do, it will prove that growth hormone function is not the whole reason for their differentiation. If growth hormone is not at all involved, the dwarfs of the three strains would be expected to maintain the same relative differences in weight as the normal mice; in other words, the proportionate effect of the dwarf gene would be the same in all three strains. If growth hormone is found to be involved to some extent in the differentiation of the strains, the next question is whether the differences are in the amount of growth hormone, or in tissue sensitivity. If it is the former, the dwarfs of the three strains will respond equally to the same dose of exogenous growth hormone; if it is the latter they will respond differently.

MATERIALS AND METHODS

The mice used in the study were representatives of the Q strain (Falconer, 1973). Briefly, the Q strain is the result of selection for high and low body weight at 6 weeks of age. The selection was replicated six times, so that there were six selected large (LA to LF), six selected small (SA to SF) and six unselected control lines (CA to CF). In the work described here, two large (LB and LD), two small (SB and SD) and two control lines (CB and CD) were used. At this point the selected lines had undergone 21 generations of selection, and the control lines 21 generations of random mating. The hypopituitary dwarf gene, *dw* was introduced into each of these six lines by successive backcrosses of heterozygous animals (*dw*/+) to the selected and control lines. After six such crosses, the residual non-Q background in each line should average only 3%. Several generations of the B and D replicates carrying the dwarf gene were raised (without further selection for body size). The experimental material was thus mice of the following lines: LB, CB, SB, LD, CD and SD of dwarf (*dw*/*dw*) and normal (+/+ and *dw*/+) phenotype.

Two series of observations were made. The numbers of mice and their treatments are given in Table 1. First, the weights of normal mice of each line were recorded up to 20 weeks of age, and the weights of dwarfs (*dw*/*dw*) with no treatment up to 28 weeks of age. Also, dwarfs from all lines were treated with growth hormone at three dose levels and their weights recorded up to 10 weeks. The doses were 1000, 500 and 250 μg per week. In the second series, dwarfs were treated with growth hormone at four lower dose levels, 125 down to 15·625 μg per week, and with saline

to provide a measure of growth with zero dose. Weights were recorded up to 14 weeks of age.

The numbers of males and females in each group were nearly equal, as were the numbers from each of the replicate lines. Though the sexes and the replicates differed in their mean weights, their differences were consistent and predictable, and they all reacted in the same way to the various treatments. They have therefore been combined and the results presented are the mean of the sexes and of the two replicates in each size-group, i.e. large (L), control (C) and small (S) lines.

Table 1. *Numbers of mice used, and their treatments. Doses of exogenous growth hormone are μg per week. L, C and S refer to the large, control and small lines*

Genotype	1st Series treatment	L	C	S	Genotype	2nd Series treatment	L	C	S
Normal	Untreated	80	80	80	Dwarf	125	19	20	20
Dwarf	Untreated	33	38	39	Dwarf	62·5	21	20	20
Dwarf	1000	6	11	11	Dwarf	31·25	20	20	20
Dwarf	500	6	12	12	Dwarf	15·625	19	20	20
Dwarf	250	12	12	12	Dwarf	Saline	20	20	20

The normal cubed diet was supplemented with crushed oats and maize which reduced the mortality of the dwarfs. In addition, the dwarfs of the first series, but not those of the second, were given 'wet mash', consisting of the cubed diet powdered and moistened with water and put on the floor of the cage. Normal mice were weaned and weighed at 3 weeks of age and thereafter caged in like-sex groups of six and weighed weekly. Dwarf mice were weighed at 3 weeks and re-weighed at about 4 weeks when they were weaned in mixed sex groups of ten. (The weaning age was in fact the Friday closest to age 4 weeks.) Treatment with growth hormone or saline was begun 3 days later (the following Monday). Hence in the dwarfs there was an age range of 25–31 days at weaning and 28–34 days at the start of the treatment.

The growth of the dwarfs between 3 and 5 weeks was very irregular, whether treated with growth hormone or not, and many dwarfs lost weight over this period. The reason for the irregular growth was the varying milk supplies of the mothers. Sometimes a new litter was born before the dwarfs were weaned, and the dwarfs then benefited from the renewed milk supply; sometimes there was no new litter and the drying off of the milk supply led to a loss of weight of the dwarfs. No adjustments were made for these varying circumstances, but in the analyses of growth we have taken 5 weeks as the starting weight from which to assess the responses to the treatments.

Bovine Growth Hormone (Calbiochem Ltd.) was solubilized in a drop of 0·1 N sodium hydroxide (BDH) and then made up to the appropriate concentration in heat sterilized physiological saline (0·9% w/v) (BDH). The growth hormone solutions were stored at +4 °C but were allowed to warm to room temperature before injection. Injections were intraperitoneal, given five times per week, and

injection volume was always 0·1 ml regardless of dose. The doses were not adjusted for the body weights of the individual mice. Heat sterilized physiological saline was used for the series of injected controls.

RESULTS

(i) *Comparisons between dwarf and normal mice*

Fig. 1 shows the growth of the normal mice and the dwarfs without exogenous growth hormone. The saline-injected dwarfs are shown by dotted lines. The weights are plotted on a logarithmic scale in order to facilitate comparisons of relative growth. The first question to be asked is: does growth hormone play any

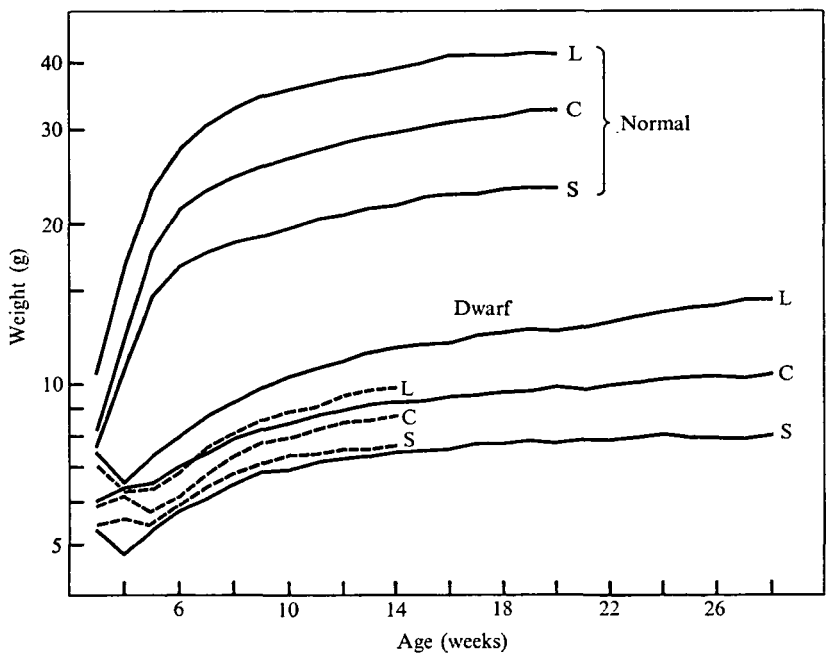

Fig. 1. Growth of normal and dwarf mice of the large (L), control (C) and small (S) strains. The untreated dwarfs are shown by solid lines, the saline-injected dwarfs by dotted lines.

part in the differentiation between the large, control and small strains? If the differences between the normal mice of the three strains were entirely due to differences in their growth hormone, then the dwarfs of the three strains would grow at the same rate since all the cause of the differences would be removed by their lack of growth hormone. If on the other hand growth hormone played no part in the differentiation of the strains then removal of growth hormone in the dwarfs would have no effect on the differences between the L, C and S strains. The dwarfs of the three strains were clearly different in weight throughout their whole growth. (The differences were less among the saline-injected dwarfs than among the untreated dwarfs; two possible reasons being the provision of 'wet mash' to the

untreated dwarfs, as noted under Methods, and the stress of the injections with saline which Bartke (1965) found reduced the growth of Ames dwarfs.) The differences of weight between the dwarfs of the three strains suggest that growth hormone cannot account for the whole of the differentiation between the L, C and S strains. The differences, however, were present already at 3 weeks and increased very little with post-weaning growth. Consequently the differences in their later weights between the dwarfs of the three strains may be largely due to their pre-weaning growth. It is therefore necessary to compare their post-weaning growth

Table 2. *Relative growth of normal and dwarf mice over different periods from 5 to 20 weeks of age. Each entry is the percentage gain in weight over the period*

		Period, weeks of age				
Strain		5–10	10–14	5–14	14–20	5–20
Normal	L	54·5	9·7	69·5	6·6	80·7
	C	50·4	12·1	68·7	9·2	84·3
	S	34·7	10·2	48·4	7·3	59·3
Dwarf, untreated	L	39·7	13·7	58·9	7·8	71·2
	C	29·2	9·5	41·5	6·5	50·8
	S	30·2	7·2	39·6	4·1	45·3
Dwarf, saline	L	39·5	10·0	53·4	—	—
	C	36·3	10·4	50·5	—	—
	S	36·1	4·4	42·1	—	—

rates. Table 2 gives the relative growth, as the percentage increase, over various periods from 5 to 20 weeks. Here we are concerned only with the dwarfs. Five independent comparisons of the L, C and S dwarfs can be made, both groups in the consecutive periods 5–10 and 10–14 weeks, and the untreated dwarfs in the period 14–20 weeks. In all the comparisons the relative growth of L is greater than S, and in three the order is L > C > S. Furthermore, in the total growth to 14 or 20 weeks the order is L > C > S in both groups. These comparisons leave no doubt that the dwarfs of the three strains differed in relative growth rate, from which it must be concluded that growth hormone cannot be the only cause of the differences between the L, C and S strains.

This leaves us with the question whether growth hormone plays any part in the differentiation of the strains. To answer this question we have to decide whether the differences between the normal L, C and S mice remain unchanged in the dwarfs. There are three ways in which this question might be approached, of which only the third gives a clear answer. The first is by consideration of Fig. 1. The distance on the graph separating the strains at any age is a measure of the relative weights of the strains. The saline-injected dwarfs show clearly that the L, C and S strains are less different when lacking growth hormone than they are in the normal mice. The strains are also less different in the untreated dwarfs than in the normals, up to about 18 weeks, but this is not easy to see by inspection of the graphs. The second way of approaching the question is by consideration of the relative growth

given in Table 2. There is a difficulty here, however, because the pattern of growth is very different in dwarfs and normal mice. The normal mice achieve most of their growth in the first few weeks, after which the strains differ little in relative growth, particularly the L and C strains. The dwarfs, in contrast, continue growing over a

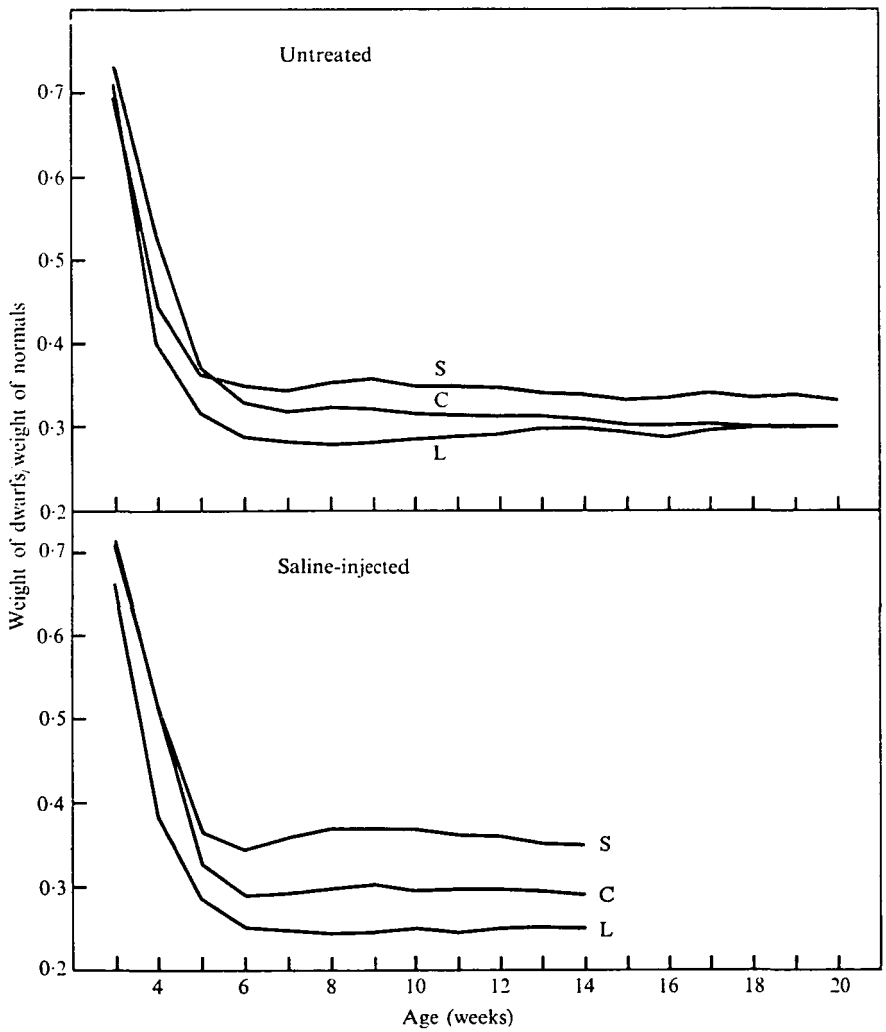

Fig. 2. Relative effects of the dwarf gene in the three strains.

longer period, and the difficulty is to know over what periods the growth of dwarf and normal mice can be compared. The third approach to the question, which gives the clearest answer, is by considering the effect of substituting the dwarf gene into the three strains. If growth hormone has some effect in making the L strain larger than the C strain, then removal of growth hormone by the dwarf gene will have more effect in the L strain than in the C. Similarly, if some form of deficiency of growth hormone makes the S strain smaller than the C then the dwarf gene will

have less effect in S than in C. The effect of the dwarf gene is assessed from the relative weight of dwarfs to normals, and this is plotted at successive ages in Fig. 2. The dwarfs start at 3 weeks by being about 70% of the weight of normals in all three strains. The effect of the gene increases sharply till 6 weeks when it stabilizes, with dwarfs between about 25 and 40% of the normals. The two groups of dwarfs agree in showing the order of effect of the gene to be L > C > S, with the exception that the effects in L and C become equal in the untreated dwarfs at the highest ages. Despite this slight inconsistency we think the conclusion that must be drawn from Fig. 2 is that growth hormone does play some part in differentiating both the large and the small strains from the control.

(ii) *Tissue sensitivity*

The next question is whether the differences between the strains lie in the amount of growth hormone or in the sensitivity of the target organs. To test for differences of sensitivity, the responses of L, C and S dwarfs to exogenous growth hormone were compared. Fig. 3 shows the growth of the dwarfs at four of the seven dose-

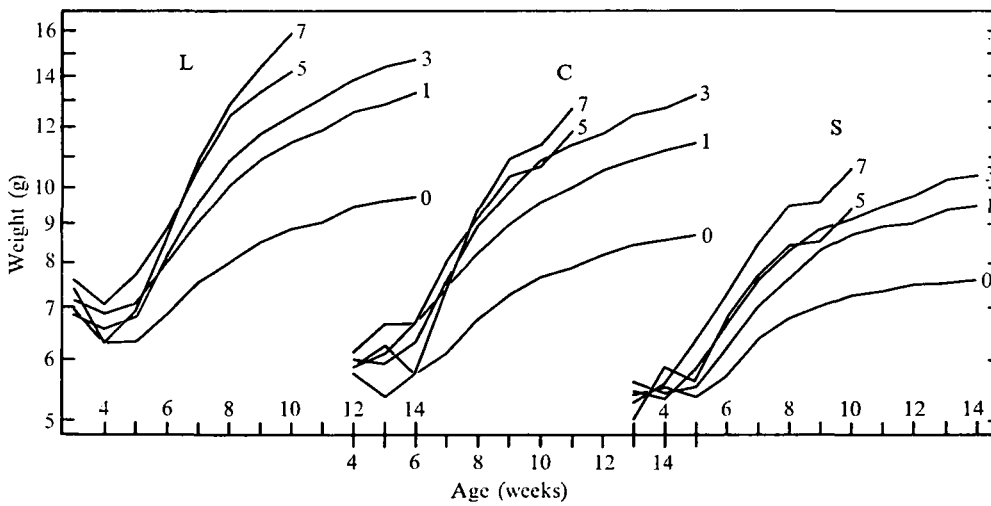

Fig. 3. Growth of dwarfs given exogenous growth hormone. Dose-levels in μg per week: 0 = saline, 1 = 15·625, 3 = 62·5, 5 = 250, 7 = 1000.

levels, and when injected with saline (dose-level 0). The three omitted dose-levels, which are used in the subsequent analyses, do not affect the picture. For the analyses, growth was assessed over the period from 5 to 10 weeks, starting at 5 weeks because of the irregularity of growth before then, as noted under Materials and Methods, and stopping at 10 weeks because doses 5, 6 and 7 were not continued beyond that age.

It is clear from Fig. 3 that the dwarfs of all three strains responded to exogenous growth hormone, and that the higher the dose the greater was their growth. The graphs suggest that the small dwarfs responded less to increasing doses than did the large and control. A proper assessment of the response, however, must take

account of the growth achieved without exogenous hormone, as shown by the saline-injected dwarfs. The arithmetic difference between the growth with hormone and the growth without gives a measure of the response in arithmetic units. These responses, given in Table 3, were in the order L > C > S at all dose-levels except the lowest. Thus it is very clear that, when given the same dose of exogenous growth hormone, the large dwarfs grew more than the controls and the small dwarfs grew less than the controls. To interpret these differences as differences of tissue sensitivity, however, would not be justified because the dwarfs of the three strains had different starting weights at 5 weeks, and their sensitivities should be assessed from their relative growth. The sensitivities can be estimated from the relative growth in the following way.

Table 3. *Responses of dwarfs to exogenous growth hormone. Growth (g) from 5 to 10 weeks with growth of the saline-injected dwarfs subtracted. Dose-levels are by two-fold increases from 15·625 μg per week at level 1 to 1000 μg per week at level 7*

	Dose-level						
Strain	1	2	3	4	5	6	7
L	1·9	2·3	3·2	4·1	4·0	5·3	6·5
C	1·2	1·7	3·0	3·3	3·1	3·9	4·9
S	1·3	1·3	1·4	2·3	1·9	2·6	2·3
	Standard errors						
L	0·34	0·34	0·33	0·39	0·60	1·66	1·33
C	0·29	0·31	0·31	0·34	0·46	0·68	0·45
S	0·27	0·26	0·20	0·25	0·49	0·33	0·32

We assume that the two components of growth, one independent of growth hormone, combine together multiplicatively. Table 3 shows that the growth of the treated dwarfs is roughly proportional to the logarithm of the dose, so we need a formulation that allows the calculation of a linear regression on log dose. This regression will provide the estimate of sensitivity.

Let w_t and w_i be the final and initial weights respectively of dwarfs treated with saline only. Then the growth in the absence of growth hormone is:

$$w_t/w_i = (1+c), \tag{1}$$

where c is the proportionate increase without growth hormone, to be estimated from the saline-injected dwarfs. Let W_t and W_i be the final and initial weights of dwarfs when treated with growth hormone at dose D. The growth is then

$$W_t/W_i = (1+c)\,(1+aD^b), \tag{2}$$

where aD^b is the proportionate increase due to the growth hormone, in which b represents the sensitivity to growth hormone and a is the proportionate increase with a dose of 1 μg per week. The response, R, to exogenous growth hormone is the growth with treatment relative to the growth without exogenous growth hormone, and is given by

$$R = \frac{W_t}{W_i} \Big/ \frac{w_t}{w_i}.$$

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Then, combining (1) and (2), we have

$$R = 1 + aD^b,$$

$$\log (R-1) = \log a + b \log D. \tag{3}$$

The sensitivity, b, can thus be estimated as the slope of the linear regression of $\log (R-1)$ on $\log D$.

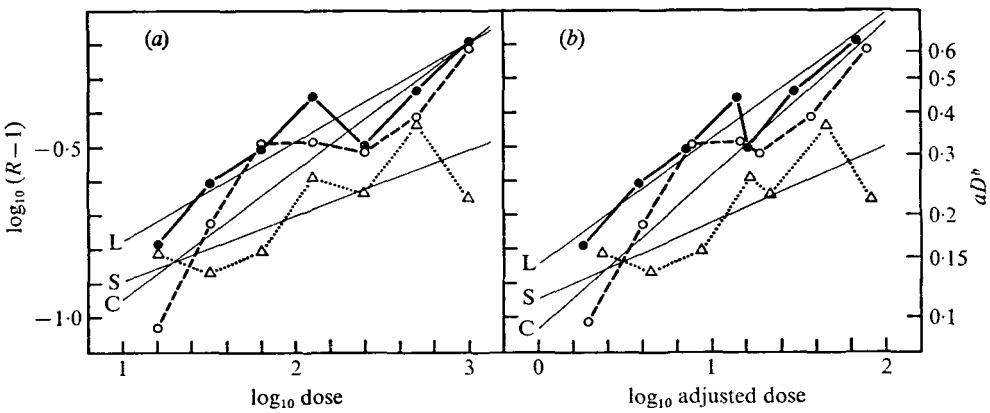

Fig. 4. Regression of log relative gain on log dose (*a*) and on log dose per gram body weight (*b*). ●, L; ○, C; △, S.

Table 4. *Regression coefficients, b, estimating sensitivity to exogenous growth hormone, by equation (3)*

	Dose not adjusted			Dose adjusted for body weight		
	L	C	S	L	C	S
c	0·39	0·36	0·36	0·39	0·36	0·36
log a	−1·07	−1·32	−1·08	−0·84	−1·03	−0·94
S.E. of log a	0·12	0·16	0·14	0·05	0·09	0·09
a	0·08	0·05	0·08	0·14	0·09	0·11
b	0·29	0·37	0·19	0·37	0·45	0·23
S.E. of b	0·06	0·08	0·07	0·05	0·08	0·08
$t_{(10)}$	0·77		1·74	0·85		2·02
P	> 0·4		0·11	> 0·4		0·07

Fig. 4(*a*) shows the responses of each of the three strains to the seven dose levels, $\log (R-1)$ being plotted against $\log D$, where D is the weekly dose in μg. The fitted regression lines are shown on the figure and the estimates of c, a and b are given in Table 4. In the calculation of the regression coefficients each point was weighted by the number of animals contributing to it. The graphs in Fig. 4(*a*), which compare the strains on the basis of their relative growth, show that the large and control strains differ little, if at all, in sensitivity, but the small strain appears to be less sensitive than the other two. Thus the conclusion from the arithmetic growth in Table 3 is borne out for the small strain but not for the large. Assuming that

relative growth is the right basis for comparison, we conclude that the large and control strains do not differ in sensitivity to exogenous growth hormone. The difference between the small and control strains can be seen in two ways. First, the response of the small dwarfs was less than that of the controls at all dose-levels except the lowest. This is not a very reliable comparison because the levels of all the points depend on the single estimate of the growth of the saline-injected mice. Second, the slope of the regression line, b, is less in the small than in the control dwarfs. This difference is significant only at the level of $P = 0{\cdot}11$. The differences of both elevation and slope, though not formally significant, strongly suggest that the small strain is less sensitive than the control to exogenous growth hormone when judged by the relative growth. The sensitivity of the small strain, estimated from the regression coefficient, is about half that of the control.

The mice, as already noted, were given a fixed dose of growth hormone, regardless of their weights. Adjustment for the differences of body weight should perhaps be made. This was done by calculating the dose per gram body weight at each week, and taking the mean of these adjusted doses. Fig. 4(b) shows the dose–response curves based on these adjusted doses. The adjustment does not affect the conclusion, but the difference between the small and control strains in the slopes of their regression lines is now nearly significant with $P = 0{\cdot}07$ (see Table 4). This, together with the difference in levels of response, leads us to believe that the lower sensitivity of the small strain is real.

DISCUSSION

The results show that selection for body weight affected growth hormone function in two ways. Selection for large size increased the 'amount' of circulating hormone, and selection for small size reduced the 'sensitivity' of the target organs. (The meaning of 'amount' and of 'sensitivity' will be considered later.) But the responses to selection were not wholly attributable to these changes. Some part of the differences in growth-rate were independent of growth hormone and were exhibited in its absence, i.e. in the dwarfs. So selection also affected some other aspect of growth control not associated with growth hormone. We would have liked to say what proportion of the total response was due to the changes in growth hormone function, but this is not possible for the following reason. In principle, equation (2) could be used to partition the total growth of normal mice into the component associated with growth hormone and the component associated with other factors. This would require the comparison of normal and dwarf mice. But the shape of the growth curves of the two is quite different; by the age of 5 weeks, when the dwarfs start to grow, the normal mice have completed the major part of their growth (see Fig. 1). So there is no period over which a meaningful comparison of growth can be made.

The effects of selection in opposite directions have been asymmetrical, indeed qualitatively different. Many examples of asymmetrical responses are known, and some of qualitatively different responses, for example in litter size (Falconer, 1960).

Apparent asymmetry, particularly of correlated responses, however, can easily arise from random drift. With only two replicates studied it is not possible to exclude random drift, and furthermore the comparisons of the large and small strains with the control were not very precise. It is possible therefore that the two aspects of growth hormone function, 'amount' and 'sensitivity', were both changed equally in the large and small strains.

There have been several previous studies in which growth hormone levels have been assayed and shown to be associated with differences of growth-rate in mice and pigs. The inbred mouse strains C3H and C57BL differ in body weight at all ages, C3H being the heavier. Yanai & Nagasawa (1968) and Sinha *et al.* (1975) both found the growth hormone content of the pituitaries to be higher in C3H than in C57BL. Baird, Nalbanov & Norton (1952) investigated two strains of Hampshire pigs that had been selected for fast and slow growth respectively. The fast growing strain had a higher level of growth hormone in the pituitaries. The authors concluded also that the potency of the growth hormone differed, that of the fast growing strain being greater. Althen & Gerrits (1976) compared both the serum and the pituitary concentrations of growth hormone in the Duroc and Yorkshire breeds of pig. They found that the faster growing Duroc breed had higher concentrations of growth hormone in the pituitaries, but the circulating levels in the serum did not differ between the two breeds.

Finally, let us consider what the changes in the 'amount' of circulating hormone and in the 'sensitivity' of the target organs may mean. In his review of genetic variation in endocrine systems, Shire (1976) shows how genetic variation may affect all of the many stages from the differentiation of the specialized endocrine cells to the reaction of the cells on which the hormone acts. What appeared in our experiments as the 'amount' of hormone could have been either the potency or the concentration of the circulating hormone. Differences of potency would presumably imply differences in the structure of the hormone molecule resulting from allelic differences at the structural locus of the prohormone. If there had been polymorphism at this locus in the original population selection would have been expected to fix the more potent allele in the large line and the less potent in the small line. The small line would then have shown a lower 'amount' of growth hormone than the control, which it did not, so a difference of potency seems rather unlikely. Differences in the concentration of circulating hormone could have been associated with differences in the number of acidophil cells in the pituitary, the rate of synthesis of prohormone, the processing to growth hormone, or the storage and the degradation of both prohormone and hormone. Differences in the concentration of circulating hormone could also have resulted from changes in other endocrine organs: the output of growth hormone is increased by thyroid and glucocorticoid hormones (Martial *et al.* 1977). The increased amount of growth hormone in the large strain was inferred from the growth of dwarfs without exogenous hormone. The difference attributed to the lack of growth hormone could have resulted from the other pituitary hormones lacking or deficient in dwarfs, i.e. prolactin or thyrotropic hormone.

What appeared in our experiments as the 'sensitivity' of the target organs could have been associated with the response of the cells, or the affinity of the receptors for bovine growth hormone. Differences of sensitivity inferred from the response to exogenous hormone could have nothing to do with the target organs, but be due to different levels of circulating exogenous hormone: the strains might differ in the rate at which they degrade bovine growth hormone.

Two forms of bovine growth hormone are known, differing in one amino acid (Seavey *et al.* 1971), which may vary in frequency between breeds. Different batches of growth hormone purchased might consequently differ in the relative amounts of the two forms. If the mice reacted differently to the two forms this might account for one feature of the dose–response experiments that has not been commented on. This is the suggestion apparent in Fig. 4 that the dwarfs responded proportionately less to the three highest doses, which were the first series of experiments, than they did to the four lowest doses in the second series.

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